Method of preparing porphyrin derivatives, porphyrin derivatives, uses thereof and pharmaceutical compositions

The present invention relates to a method of preparing porphyrin derivatives starting from a meso-substituted porphyrin compound.

Such a method is known in the art. In particular Arnold,
5   D. et al; J. Chem. Soc. Perkin 1, pp. 1660-1670 (1978) disclose the formation of benzochlorins starting from a meso-substituted acrylalcohol- or acrylaldehydeporphyrin compound.
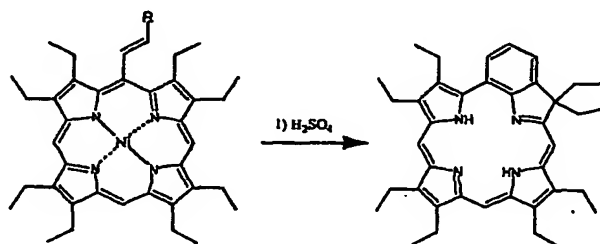
R= CH=O or   benzochlorin
· CH$_2$-OH

10   An important aspect is that such compounds can be prepared starting from hemin and protoporphyrin, which are relatively inexpensive and commercially available in relatively large amounts. This does not apply for many other porphyrins
15 · described in the literature and restricts the application thereof significantly. The method disclosed by Arnold is useful for the preparation of modified porphyrin compounds having an absorption maximum (λmax) that is shifted towards the red relative to the starting porphyrin.
20   For many applications of porphyrins it is important to have at one's disposal porphyrin derivatives having a λmax in a particular wavelength range. An example thereof are photosensitizers which are used for photodynamic therapy or for the disinfection of blood products. Hence, it is important to
25 have at one's disposal a wide range of chemical reactions for the synthesis of such porphyrin compounds starting from another porphyrin compound, such as, but not limited to, hemin

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and protoporphyrin.

The object of the present invention is to provide a new
method for the preparation of porphyrin derivatives having a
modified λmax. The object of the present invention is to also
5   provide a method for the preparation of porphyrin derivatives
possessing an increased hydrophilic nature, wherein said por-
phyrin derivatives consequently may be more suitable for
pharmaceutical applications.

To this end the method according to the invention is
10   characterized in that a meso-(2'-cyanovinyl)-substituted por-
phyrin compound of which the vinyl is optionally substituted
is used as the meso-substituted porphyrin compound, wherein
said meso-(2'-cyanovinyl)-substituted porphyrin compound, in
a form in which its porphyrin macrocycle is complexed with a
15   bivalent metal ion

        i) is subjected to

            an acid for which $0 < pKa < 5$
            and
            an oxidising agent,
20   with the restriction that if the carbon atom of the por-
            phyrin macrocycle at which the (2'-cyanovinyl) substitu-
            ent is attached is designated Cα, there must be a sub-
            stituent attached to Cδ, counting along the perimeter of
            the porphyrin macrocycle, said substituent comprising a
25   -C-C motif directly attached at the Cδ carbon atom;
        or

        ii) is subjected under aprotic conditions to a Vilsmeier
            reagent having a reactive motif

                        C^1

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                    $C^2 - N = C^3$

        containing a quaternary nitrogen atom which is directly
        linked to two carbon atoms C^1, C^2 wherein said carbon at-
        oms are not part of a unsaturated or aromatic moiety,
35   and which quaternary nitrogen atom is directly linked to
        a carbon atom C^3 via a double bond, said carbon atom C^3
        carrying a halogen atom chosen from fluoro, chloro,
        bromo and iodo

with the restriction that if the carbon atom of the por-
phyrin macrocycle at which the (2'-cyanovinyl) substitu-
ent is attached is designated Cα, there must be a sub-
stituent attached to Cδ, counting along the perimeter of
5   the porphyrin macrocycle, said substituent comprising a
-CH motif directly attached at the Cδ carbon atom;
to convert said meso-(2'-cyanovinyl)-substituted porphyrin
compound into a porphyrin derivative having a quinoline-ring
system peri-condensed to the porphyrin ring, and optionally
10   the bivalent metal ion is removed or replaced by another
metal ion, and optionally the nitrogen atom of the quinoline-
ring system ring is quaternized.
      Surprisingly, this method results in the formation of
porphyrin derivatives having a ring system with two aromatic
15   rings fused to the porphyrin macrocycle, in particular peri-
annulated quinoporphyrins, which are entirely new compounds.
These new compounds possess an aromatic nitrogen atom which
contributes to the amfiphilic nature of the new compounds
compared to porphyrin compounds known in the art. This helps
20   to increase solubility in polar solvents, such as aqueous so-
lutions, and in particular in bodily fluids such as blood.
The oxidising agent in the alternative reaction step i) is
conveniently oxygen, for example from the air. The particular
acid to be used depends on the particular substituents of the
25   vinyl group of meso-(2'-cyanovinyl), and a suitable acid can
be found without undue effort using routine experimentation
by starting with acids having different pKa's in the range of
0 to 5. Strong acids (pKa < 0) are to be avoided before the
reaction is complete because the yield will decrease due to
30   demetallation of the porhyrin macrocycle and hydrolysation of
the nitril of the meso-(2'-cyanovinyl) group. For the sake of
convenience, in the present description the term meso-(2'-
cyanovinyl) also includes derivatives of this group where vi-
nyl carries a substituent on one or both carbon atoms of vi-
35   nyl, even if this has not been mentioned explicitly or if the
term is part of a structural formula, which has been done for
the purpose of readability only. The Vilsmeier reagent of al-
ternative step ii) is conveniently prepared in situ starting

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from a carbonyl amide, for example using POX_3 where X is a halogen atom, such as chlorine. Other reactions for preparing Vilsmeier reagents are known in the art and do not form part of the invention. The reaction with the Vilsmeier reagent may

5 be performed at room temperature, whereas the reaction according to step i) will generally be performed at an elevated temperature.

The method according to the present invention was shown to be very suitable for the preparation of porphyrin deriva-

10 tives wherein for alternative step i) a meso-(2'-cyanovinyl)-substituted porphyrin compound of formula (I) is used as the starting compound,

(I)

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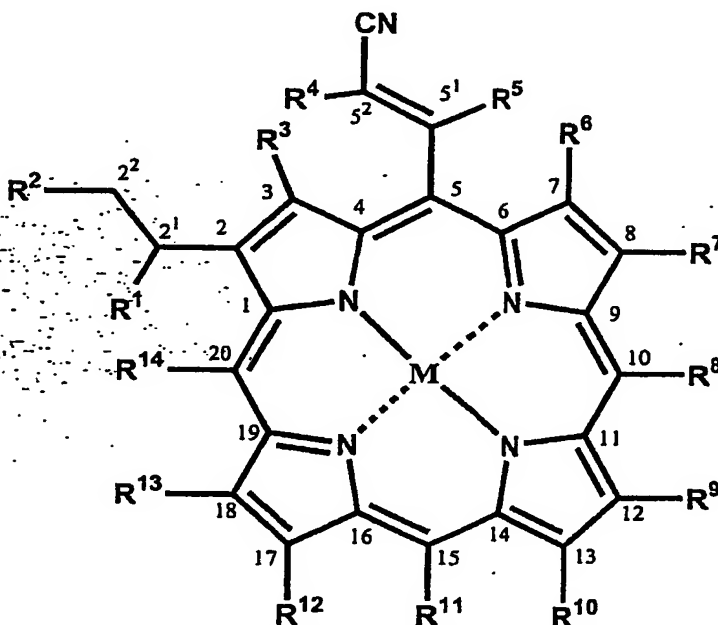
or wherein for alternative step ii) meso-(2'-cyanovinyl)-substituted porphyrin compound of formula (III) is used as the starting compound:

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(III)
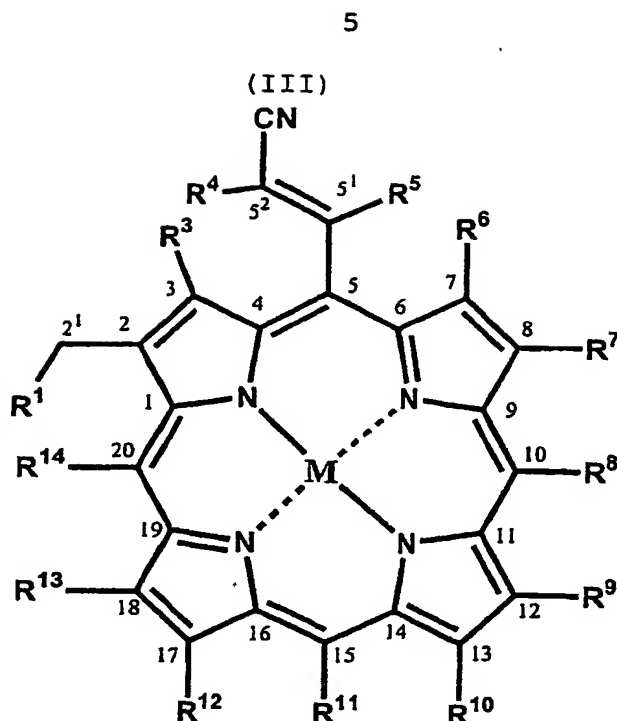
wherein

R^1, R^2 represent independently of each other hydrogen, linear or branched (C_{1-8}) alkyl, or linear or branched (C_{1-8})alkyl C(O)O (C_{1-8})alkyl, wherein the groups comprising alkyl may optionally be substituted with fluoro, chloro, bromo, iodo, nitrile, (C_{1-8}) thioether, and (C_{1-8}) alkoxy;

R^3 represents H or (C_{1-8}) alkyl;

R^4 and R^5, represent, independently of each other, hydrogen, nitrile, monocyclic, bicyclic or tricyclic (C_{6-14}) aryl, or (C_{1-4}) alkyl wherein the aryl and alkyl group may optionally be substituted with fluoro, chloro, bromo, iodo, nitrile, (C_{1-8}) thioether, and (C_{1-8}) alkoxy;

R^6 to R^{14} represent independently of each other, hydrogen, linear or branched (C_{1-8}) alkyl, linear or branched (C_{1-8})alkyl C(O)O (C_{1-8})alkyl, wherein n is an integer of 0 to 4, $CH_2=CH-$, a monocyclic, bicyclic or tricyclic (C_3-C_{14}) aryl, which aryl may optionally contain one or more nitrogen atoms as heteroatoms; and R^8, R^{11}, and R^{14} may in addition represent an acrylonitrile group substituted with $R^{4\prime}$ and $R^{5\prime}$, wherein $R^{4\prime}$ and $R^{5\prime}$ are as defined for R^4 and R^5;

and

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M represents a bivalent metal ion,
wherein the compound of formula (I) or (III) is converted
into the corresponding porphyrin derivative of formula (II)
comprising a quinoline-ring system fused to the porphyrin
5   ring

(II)
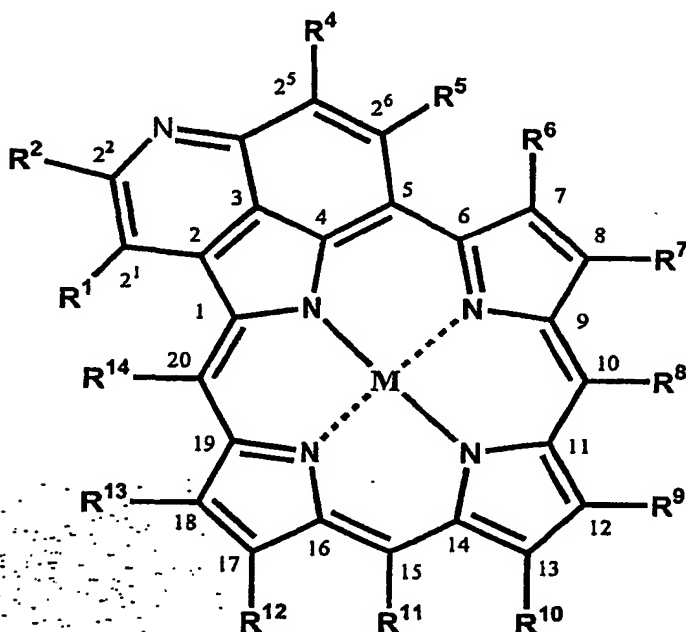
wherein the substituents have the meanings given above,
and depending on the meaning of R^8, R^{11}, and R^{14} and the corre-
10   spondence of an adjacent R^7, R^9, R^{10}, R^{12}, and R^{13} with R^3, op-
tionally more than one quinoline-ring system peri-condensed
to the porphyrin ring is present.

In the method according to the invention the meso-(2'-
cyanovinyl)-substituted porphyrin compound of formula (I) is
15   preferably prepared by introducing a formyl or acetyl residue
at a meso position of a porphyrin compound, after which the
thus formed mesoformylporphyrin or mesoacetyl porphyrin is
converted into its meso-(2'-cyanovinyl) derivative or meso
(2'-cyano-1'-methylvinyl) derivative, respectively. According
20   to a preferred embodiment, the mesoformylporphyrin formed is
converted into the meso-(2'-cyanovinyl)-substituted porphyrin
compound of formula (I) by reaction with diethylphosphonoace-

tonitrile. Other reactions are easily available in accordance
with the invention, for example with malononitrile ($NCCH_2CN$)
to result in a meso-(2',2'-dicyanovinyl)-substituted porphy-
rin compound where R^4 is nitrile. Thus, the starting mate-
rial, the meso-(2'-cyanovinyl) compound of formula (I), for
performing the method according to the invention is obtained
in a simple manner. Usually the porphyrin compound used as a
starting material will contain a hydrogen atom at at least
one meso position (i.e., at least one of R^8, R^{11} and R^{14} is hy-
drogen).

In case a substituent R^4 or R^5 is an aryl group, the aryl
group is usually phenyl, naphthyl, phenanthryl or anthracyl.
The ester groups, such as those of R^{10} and R^{12}, may optionally
be hydrolysed to yield carboxylic acids, further improving
the solubility of the present porphyrin derivatives in aque-
ous solutions. These groups also provide excellent starting
points to couple the porphyrin derivatives to other mole-
cules, such as proteins, or substrates using methods for cou-
pling well known in the art. Also, is it possible to attach
one or more groups to the porphyrin derivative, for example
to one or more carboxylic acid groups obtained as described
above. For example, to improve solubility in aqueous solu-
tions a group comprising a PEG tail may be introduced in the
porphyrin derivative. Alternatively, more charged groups may
be introduced.

According to a very favourable embodiment, the nitrogen
atom of the peri-condensed quinoline-ring system in formula
(I) is quaternized.

This results in porphyrine compounds that have an im-
proved solubility in water thanks to the positive charge on
the quaternary nitrogen atom(s), as a result of which the
solubility is less dependent on pH. Even more interesting,
such porphyrin derivatives have absorption maxima in the far
red (say 750 nm). The group substituting the nitrogen atom(s)
is preferably alkyl and aryl as defined for R6 but may also
be, for example, alkaryl such as benzyl and optionally sub-
stituted as for R6. The particular choice will depend on the
desired nature of the porphyrin derivative.

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An advantageous embodiment is characterized in that the porphyrin compound used as a starting material for the preparation of the meso-(2'-cyanovinyl) porphyrin is chosen from the group of i) hemin, and ii) heme.

5   The synthesis of porphyrin derivatives, in particular on a large scale (hundred grams or more) and with high purity has been a problem until now. The simple chemical reaction method according to the invention makes it possible to obtain porphyrin derivatives with excellent yield and good purity

10   after limited purification.

To perform a reaction according to the invention it is preferred that Ni^{2+} is used as the bivalent metal ion.

This was shown to result in good yields. If desired, the metal ion may be removed (by introducing two hydrogen atoms)

15   or replaced after reaction, using methods well known in the art (References: Fuhrop, J.H. et al in *Porphyrins and Metalloporphyrins*, Smith, K.M.; Ed Elsevier: Amsterdam, 1975; p. 185 and pp. 795-798. Buchler, J.W.; In *The Porphyrins*; Dolphin, D.; Ed.; Academic Press, New York 1978, Vol 1A, p. 389.

20   Sanders et. al.; In *The Porphyrin Handbook*, Kadish, K.M., Smith, K.M. and Guilard, R.; Ed.; Academic Press, San Diego, 2000, Vol 3, Chapter 15, pp. 3- 40. demetallation of porphyrins: Fuhrop, J.H. et al in *Porphyrins and Metalloporphyrins*, Smith, K.M.; Ed Elsevier: Amsterdam, 1975; pp. 195-207 and

25   pp. 243-247. Particular reference is made to Buchler, J.W. et al, *Liebigs annalen der Chemie* (1988), pp. 43-54).

To achieve good yields and purities, it is preferred to perform the reaction according to the invention with a Brönsted-acid, using a Brönsted-acid with the provisio that 0 <

30   pKa < 5, at a temperature above 140°C.

Lower temperatures, for example 90°C may also be employed successfully, depending on the particular nature of the substituents involved in the reaction. In general, pKa values between 0.5 and 2.5 are preferred, but again this will

35   depend on the particular nature of the substituents involved in the reaction. It is well within the skill of an ordinary person skilled in the art to determine suitable reaction conditions using routine experimentation using the disclosed ex-

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amples as guidance.

Preferably the Vilsmeier reagent used is of formula (IV)

(IV)

$$R_{15}\diagdown \underset{\oplus}{\overset{}{N}}=\overset{R_2}{\underset{X}{\diagup}}$$
$$R_{16}\diagup$$
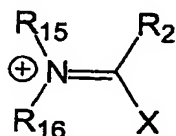
wherein

R15 and R16 are, independently of each other, linear or branched C_{1-8} alkyl,

X is fluoro, chloro, bromo and iodo, and

R2 is hydrogen, linear or branched (C_{1-8}) alkyl, or linear or branched (C_{1-8}) alkyl $C(O)O$ (C_{1-8}) alkyl, wherein the groups comprising alkyl may optionally be substituted with fluoro, chloro, bromo, iodo, nitrile, (C_{1-8}) thioether, and (C_{1-8}) alkoxy.

Such Vilsmeier reagents are sufficiently electrophilically "hard" to obtain the desired quinoporphyrins. It is preferred that X is chloro or bromo.

In addition the invention relates to every porphyrin derivative having a quinoline-ring system peri-condensed to the porphyrin ring, and more specifically a porphyrin derivative having a quinoline-ring system peri-condensed to the porphyrin ring obtainable with the method according to the invention. Please note that this includes derivatives having more than one quinoline-ring system.

More specifically the invention relates to porphyrin derivatives, wherein said derivatives are:

- 2'-methoxycarbonylquino[4,4a,5,6-jkl]-annulated 12-demethyl-13-de[2-(methoxycarbonyl)ethyl]mesoporphyrin dimethylester;

- 2'-methoxycarbonylquino[4,4a,5,6-qrs]-annulated 18-demethyl-17-de[2-(methoxycarbonyl)ethyl]mesoporphyrin dimethylester;

- quino[4,4a,5,6-abt]-annulated 2-demethyl-3-deethylmesoporphyrin dimethylester;

- quino[4,4a,5,6-efg]-annulated 7-demethyl-8-
deethylmesoporphyrin;

- 2'-methoxycarbonylquino[4,4a,5,6-jkl]-annulated 12-
demethyl-13-de[2-(methoxycarbonyl)ethyl]mesoporphyrin;

5   - 2'-methoxycarbonylquino[4,4a,5,6-qrs]-annulated 18-
demethyl-17-de[2-(methoxycarbonyl)ethyl]mesoporphyrin;

- quino[4,4a,5,6-abt]-annulated 2-demethyl-3-
deethylmesoporphyrin;

- quino[4,4a,5,6-bcd]-2-demethyl-3-deethyl-mesoporphyrin
10   dimethylester;

- quino[4,4a,5,6-bcd]-2-demethyl-3-deethyl-
mesoporphyrin;

- 3'-methylquino[4,4a,5,6-efg]-7-demethyl-8-
deethylmesoporphyrin dimethylester;

15   - 3'-methylquino[4,4a,5,6-efg]-7-demethyl-8-
deethylmesoporphyrin;

- 9'-aminocarbonylquino[4,4a,5,6-efg]-7-demethyl-8-
deethylquinoporphyrin dimethylester;

- 9'-aminocarbonylquino[4,4a,5,6-efg]-7-demethyl-8-
20   deethylquinoporphyrin

- N-benzylquinolinium[4,4a,5,6-efg]-annulated mesopor-
phyrin dimethylester

- N-benzylquinolinium[4,4a,5,6-efg]-annulated mesopor-
phyrin.

The invention also contemplates an (optionally substi-
tuted) meso-(2'-cyanovinyl)porphyrin useful as a starting ma-
25   terial for the preparation of a corresponding quinoline-ring
systemporphyrin derivative. Optionally substituted refers to
both vinyl and the porphyrin macro-cycle.

Finally, the invention relates to the use of a porphyrin
derivative according to the invention for the preparation of
30   a pharmaceutical composition for treating those indications
that are well known in the art for clinical and other uses of
photosensitizers. These indications include amongst others:

1) Skin and mucosa disorders: benign, malignant, in-
flamed and infectious skin/mucosa disorders such as acne,
35   warts, eczema, birthmarks (including vascular malformations
such as naevus flammeus and hyperpigmentation), hirsutism,

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skin/(burn)wound/mucosa infections (caused by bacteria, vi-
ruses, dermatophytes and other fungi, yeasts and/or para-
sites), actinic keratoses, psoriasis, primary tumors (includ-
ing basal cell carcinomas, squamous cell carcinoma and mela-
5 nomas) and secondary tumors of the skin and mucosa. In addi-
tion, photosensitizers can be used to decontaminate the skin
and mucosa for the prevention of infections;

2) Vascular disorders: vascular diseases such as the
different kinds of macula degeneration in ophthalmology,
10 treatment of atherosclerotic plaques, prevention and/or
treatment of vascular (re)stenosis or aneurysms, arterio-
venous malformations and other vascular anomalies;

3) Oncology: as an alternative or addition to the stan-
dard treatment of tumors and pre-cancerous lesions such as
15 pancreas head cancer, tumors of the brain, lung, cervix,
uterus, urinary bladder, bile bladder, stomach, gut, thyroid
and oesophagus (including Barret's oesophagus), prostate can-
cer, head and neck cancers (including cancers of the oral
cavity, ears, nose, larynx and pharynx) and kidney tumors;
20 4) Ophthalmology disorders: disorders in the eye such as
age-related macula degeneration, secondary cataract, infec-
tions, immunological diseases and tumors;

5) Gynecological or urological disorders: urogenital
diseases such as uterus bleedings, endometriosis, benign
25 prostate hypertrophy and for use in endometrial ablation;

6) Immunological disorders: diseases caused by aberra-
tions of the immune system or increased inflammatory reac-
tions such as multiple sclerosis, rheumatoid arthritis, In-
flammatory Bowel Disease (including colitis ulcerosa and
30 Crohn's disease), scleroderma and thyroiditis;

7) Oral cavity or nasopharyngeal disorders, including
dentistry applications, for example disorders in the oral
cavity such as decontamination of root canals, treatment
and/or prevention of gum disease and treatment of wounds or
35 other mucosal disorders.

The porphyrin derivative in a pharmaceutical composition
according to the invention may be present in any suitable
form, including as its acid or basic addition salt or the

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free base and free acid thereof and the pharmaceutical compo-
sition will generally include a pharmaceutically acceptable
carrier or excipient. In general, porphyrins not containing a
metal ion will be preferred.

5   In addition, the derivatives according to the present
invention are useful

          1) for photodetection of malignant and pre-malignant le-
sions for instance in the bladder, lung or esophagus;

          2) for decontamination or pathogen (such as gram posi-
10   tive and negative bacteria, viruses, parasites, prions and
fungi) reduction of liquids such as biological fluids (in-
cluding donor blood, stem cell containing fluids, bone marrow
purging) and contaminated water;

          3) for decontamination or pathogen reduction of surfaces
15   either by using liquid photosensitizers or by coupling them
directly to the said surface;

          4) for use as insecticide.

          The appended claims are included in the description by
reference.

20   The invention will now be illustrated with reference to
the following examples, and with reference to the drawing,
wherein

          Scheme S1 depicts the conversion of mesoporphyin dimeth-
ylester to the corresponding acrylonitrile derivative via the
25   formyl derivative; (I = nickel (II) acetate tetrahydrate, di-
methylformamide, reflux; II = methylformanilide, phosphorus
oxychloride, dichloroethane, RT.; III = diethyl phosphono
acetonitrile, sodium hydride, tetrahydrofuran, reflux);

          Scheme S2 depicts the conversion of the acrylonitrile
30   derivative of scheme 1 to the peri-condensed quinoporphyrin
according to the invention (IV = trichloroacetic acid, 175°C,
2 minutes; V = concentrated sulfuric acid, RT.);

          Scheme S3 depicts the proposed reaction mechanism of the
conversion of the acrylonitrile derivative of scheme 1 to a
35   peri-condensed quinoporphyrin according to the invention us-
ing an acid;

          Scheme S4a and S4b depict the proposed reaction mecha-
nisms of the conversion of the acrylonitrile derivative of

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scheme 1 to peri-condensed quinoporphyrins (8 and 9) accord-
ing to the invention using a Vilsmeier reagent.

Fig. 1 shows the photodynamic activity of a derivative
according to the invention; and

5   Fig. 2 shows the fast clearance of a porphyrin deriva-
tive according to the invention in mice.

EXAMPLE 1
        Step A

10   Protoporphyrindimethylester (Sato Pharmaceuticals Ltc,
Tokyo, Japan) was used for the preparation of mesoporphyrin-
dimethylester (1) according to the method disclosed by
Fuhrop, J.H. et al (Porphyrins and Metalloporphyrins, supra).
110 g (0,18 mole) of mesoporphyrindimethylester (1), dis-

15   solved in 1,5 l of dimethylformamide containing 0,2 mol of
nickel (II) acetate, was refluxed for 15 minutes. After
evaporation of the solvent under vacuum at 80°C, the subse-
quent chromatography on silica yielded 103 grams (0,16 mol,
86%) of the mesoporphyrindimethylester-nickel complex 2.

20
        Step B
        A Vilsmeier-formylation was conducted with methylfor-
manilide and POCl₃ in 1,2-dichloroethane (Vilsmeier, A. et
al, Ber. 60, p. 119, (1927); Minkin et al., Chem. Rev. 74, p.

25   87-99 (1974)). This yields a mixture of monoformyl deriva-
tives 3a - 3d.

        Step C
        The mixture of four monoformyl derivatives 3a - 3d ob-

30   tained in Step B was converted into the corresponding meso-
acrylonitrile derivatives 4a - 4d via a Horner-Emmens reac-
tion (Van den Berg, E.M.M. et al Recl. Chim. Trav. Pays-Bas,
109(3), p. 160-167 (1990), Boutagy et al. Chem. Rev. 74, p.
87-99, (1974)) with the anion of diethylphosphonoacetonitril.

35   The crude mixture was first separated into two fractions by
silicagel chromatography using dichloromethane as the eluent.
The first fraction (20g) mainly contained 4a, but also 4b and
4d in a 3:1:2 ratio. The second fraction (57 g) contained all

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isomers 4a, 4b, 4c and 4d. Fractional crystallisation of the
first fraction from a mixture of dichloromethane and hexanes
by slow evaporation of dichloromethane yielded 6,6 grams of
pure isomer 4a as a bright red solid. The mother liquor con-
5   tained mainly 4b and 4d and was added to the second fraction.
Crystallisation of this mixture gave 63 grams of a mixture of
4b and 4d (ratio 1:2) as a dark green or black crystalline
material. The mother liquor contained a mixture of 4a, 4b, 4c
and 4d. From NMR it can be seen that in the crude product
10 mixture contained the four isomers 4a, 4b, 4c and 4d in a ra-
tio of 4a:4b:4c:4d=1:1:0.4:2. This ratio corresponds to what
was expected. Introduction of a formyl group at position 20
(3d) experiences the least amount of sterical hindrance be-
cause that is where the smallest (methyl) groups are. During
15 the formation of 3a and 3b the sterical hindrance is caused
by one methyl group and a larger ethyl group, whereas the
formation of 3c involves a more restricting sterical interac-
tion with two ethyl groups...

20   Step D (according to the invention)
        Refluxing of pure 4a in trichloroacetic acid at 175°C
leads surprisingly to a new peri-annulated quinoporphyrin-
product (5a). The oxidising agent performing the final oxida-
tion (closing the heterocyclic ring of the quinoline-ring
25 system) to result in the new product is oxygen from the air,
similar to what is known for other reactions involving por-
phyrins (see Woodward, J. Am. Chem. Soc. (1960) 38, pp. 3800-
3802). The λmax of this new compound 5a is, compared with
mesoporphyrindimethylester 1, shifted towards the red (re-
30 spective values for λmax are: 567 nm and 638 nm).
   Treatments of the mixture of 4b and 4d yielded a mixture
of 5b (from 4b) and 5c and 5d (from 4d).
        If desired the metal ion used (here nickel) may be re-
moved in a conventional manner (5 min. conc. sulphuric acid
35 at room temperature, the time required to dissolve com-
pletely), yielding the corresponding compounds 6a - 6d. The
full names are shown in table 1. Noteworthy are the maximum
absorption wavelengths for 6a, 6b, 6c and 6d which are 681,

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688, 688 and 683 nm respectively (in both dichloromethane and
methanol),which are very long for entirely unsaturated por-
phyrin ring systems. Benzylation of the quinoline-ring system
nitrogen with benzyl bromide/ potassium iodide leads to for-
5   mation of a cationic benzylated quinoporphyrin (**18** N-
benzylquinolinium[4,4a,5,6-efg]-annulated mesoporphyrin di-
methylester with an absorption maximum at 755 nm. Hydrolysa-
tion of this compound yields N-benzylquinolinium[4,4a,5,6-
efg]-annulated mesoporphyrin (**19**).

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        <u>5a</u> : M = Ni   <u>5b</u> : M = Ni
        <u>6a</u> : M = 2H   <u>6b</u> : M = 2H

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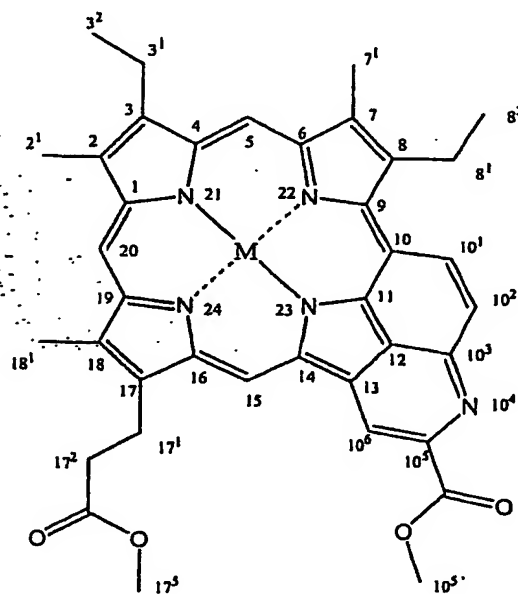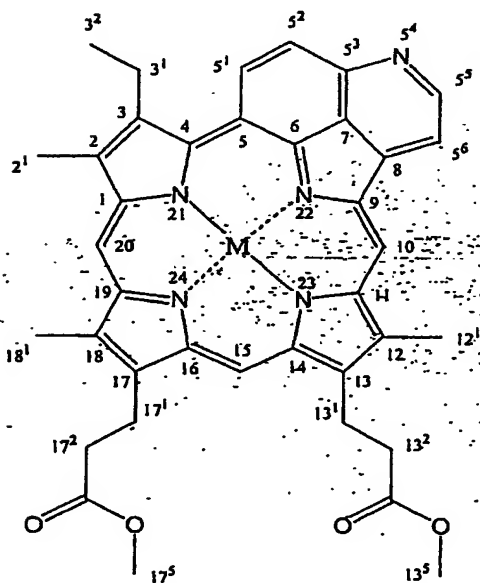
5c : M = Ni
6c : M = 2H
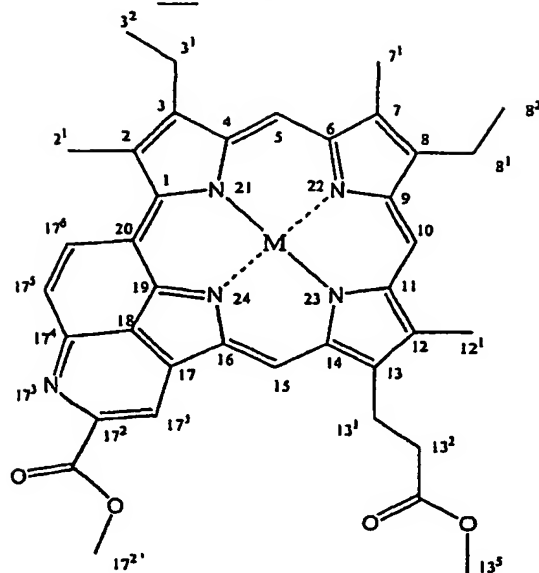
5d M = Ni
6d M = 2H
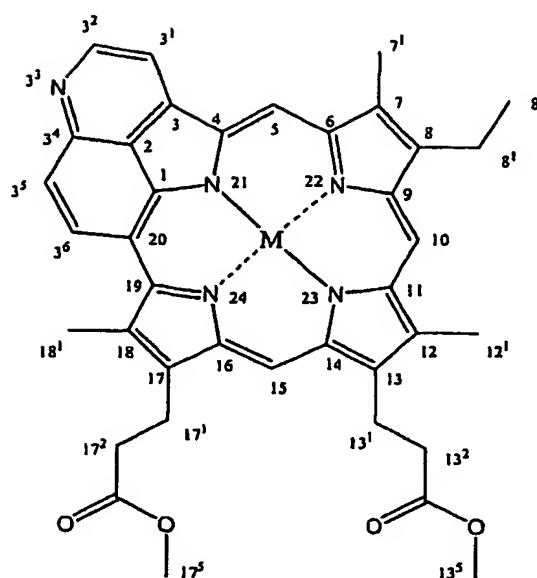
Step E (hydrolysis)

5 A solution of 0.61 gram of 6a in 25 ml tetrahydrofuran (THF)
was mixed with a solution of 0.20 gram sodium hydroxide in
120 ml of water and refluxed during 1 hour. The reaction was
followed with silicagel thin layer chromatography using THF
as the eluens. When the reaction is finished THF and water
10 were removed by distillation under reduced pressure until the
volume was approximately 20 ml. The crude product was puri-
fied over 10 grams of CM Sephadex cation exchanger (bead size
40-120 µm) from Sigma-Aldrich, which was first treated with 1
M hydrochloric acid and then washed with demineralized water.
15 First the porphyrin solution was carried onto the sephadex
gel, and washed with demineralized water to remove salts. The
purified porphyrin was eluted with 1% ammoniumhydroxide in
water. The resulting brown solution was evaporated to dry-
ness, then demineralized water was added and again the solu-
20 tion was evaporated to dryness to remove traces of ammonia.
This was repeated two times until the porphyrin does not com-
pletely dissolve in demineralized water (which is slightly
acidic). Yet this solubility of about 0.5 mg/ml at about pH 8
is better than for other porphyrin compounds such as MTHPC

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which is currently used in photodynamic therapy. 0.45 grams
(77%) of the dicarboxylic acid 7a was obtained.

(7a)
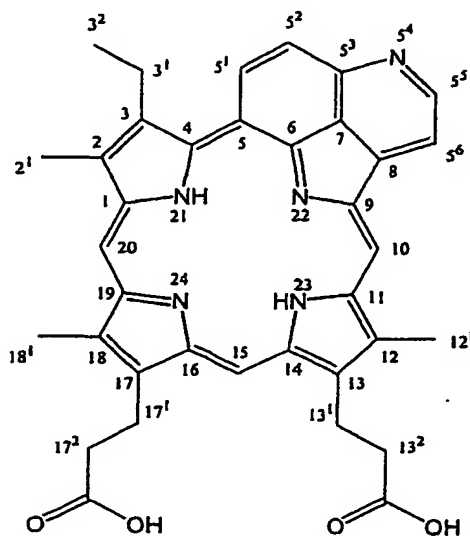
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EXAMPLE 2

Step F

The product mixture obtained from the Vilsmeier reaction on
10   400 gram of 2 has been separated into three fractions using
silicagel chromatography with dichloromethane as the eluens.
The first fraction contained 25 grams of a mixture of 3a
(75%), 3b (8%) and 3d (17%). Crystallization by slow evapora-
tion of dichloromethane from a mixture of dichloromethane and
15   ethanol yielded 12.1 grams (17.8 mmol) of 5-
formylmesoporphyrin dimethylester nickel(II) complex 3a.
This compound was used for the synthesis of 10.
(The second fraction contained 42.0 gram of a mixture of 3a,
3b and 3d in a ratio of 3:1:2 and was used for the synthesis
20   of compounds 4a, 4b, 4c and 4d.)

Step G

1.02 gram (1.50 mmol) of 3a was dissolved in a mixture of 50
ml dimethylformamide, 50 ml pyridine and 4.0 ml of malononi-
25   trile. The mixture was refluxed at 140°C during 4 hours after

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which the solvent was evaporated under reduced pressure. The crude 10a 5-(2',2'-dicyanovinyl)mesoporphyrin dimethylester nickel(II) complex was purified over silicagel using a mixture of 1% of methanol in dichloromethane. This yielded 1.03
5   gram (94%) of pure 10.

Step H
Reaction of 950 mg (1.31 mmol) of 10 followed by work-up has proceeded using the procedure described in Step C yielded 96
10   mg (0.132 mmol, 10%) of the 9'-aminocarbonylquino[4,4a,5,6-efg]-annulated 7-demethyl-8-deethylmesoporphyrin dimethylester nickel(II) complex 11.

The cyclization reaction with the Vilsmeier reagent pre-
15   pared from dimethylformamide is similar to the cyclization reaction with trichloroacetic acid. The initial step is attack at the nitrogen of the nitrile function, followed by a series of steps that lead to the quinoline formation.
          Without wishing to be bound by any particular theory,
20   our experiments to investigate the reaction mechanism made us to believe that the localized positive charge in the Vilsmeier reagent makes it a hard electrophile which makes it have a preference for the hard nitrogen atom of the nitrile function. This also takes place when an acid is used for the
25   cyclization reaction.
          In the case of the cyclization with the Vilsmeier reagent obtained from dimethylformamide two peri-condensed quinoporphyrins are formed. After attack on the nitrile-nitrogen of the Vilsmeier reagent, the carbenium ion A is formed
30   (scheme 4). Quinoporphyrin 8, which is the main product, is formed via attack of this carbenium ion at carbon 3 of pyrrole-ring A, while the minor product 9 is formed via attack on carbon 7 of pyrrole-ring B.
          Here, the carbon atom at position 5 which carries the
35   acrylonitril substituent is designated Cα and the carbon atoms at the positions 2 and 8 are Cδ. When the starting material is 4a, it can be seen   that in contrast to the quinoline-ring system formation under acid conditions (Scheme S3)

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the Vilsmeier reagent now contributes a carbon atom that is
incorporated into the quinoline system), leading to the for-
mation of two quinoporphyrins 8 and 9 (i.e. the porphyrin has
two Cδ atoms; Schemes 4a and 4b), whereas under Brönsted acid
conditions only one quinoporphyrin is formed.
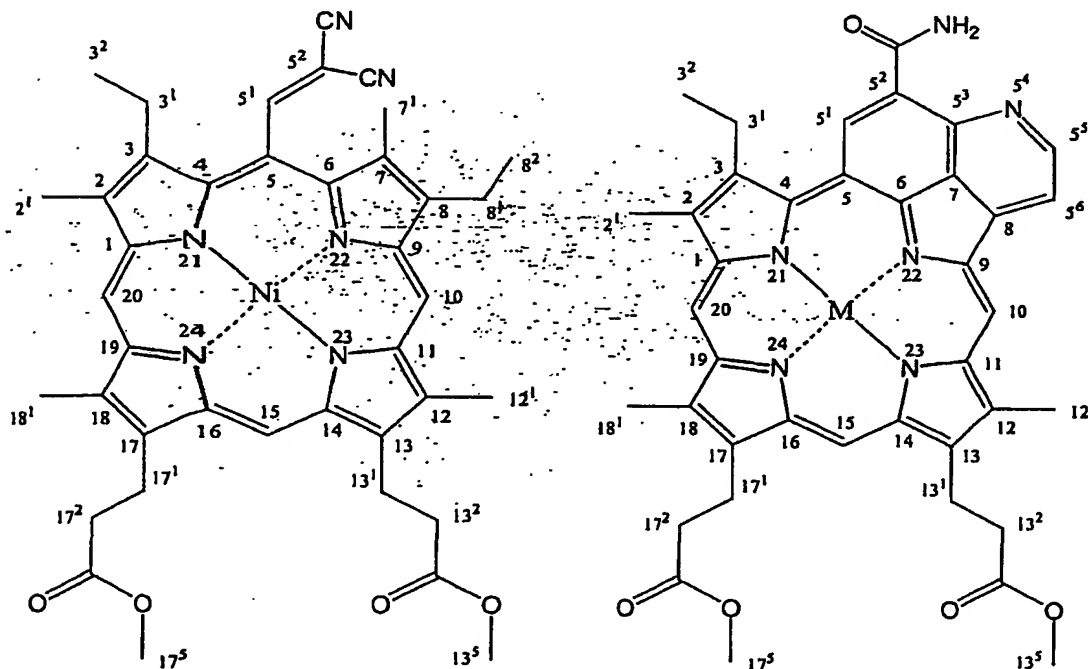
Step I
Demetallation of <u>11</u> (90 mg, mmol) following the procedure as
described in Stap D yielded 67 mg (0.10 mmol, 81 %) of the
free base <u>12</u> 9′-aminocarbonyl-quino[4,4a,5,6-efg]-annulated
7-demethyl-8—deethylmesoporphyrin dimethylester.

<u>10</u>   <u>11</u> : M = Ni
                                            <u>12</u> : M = 2H

EXAMPLE 3

Quino[4,4a,5,6-bcd]-2-demethyl-3-deethylmesoporphyrin dimeth-
ylester nickel complex (8) and 3′-methylquino[4,4a,5,6-efg]-
7-demethyl-8—deethylmesoporphyrin dimethylester nickel com-
plex (9)

a) Preparation of Vilsmeier reagent

POCl$_3$ (0.40 ml, 4.3 mmol) was added dropwise to an ice-cold
mixture of dimethylformamide (0.55 ml, 4.5 mmol) and 1.0 ml
of chloroform, after which the mixture was stirred for 20

5   minutes at room temperature.

b) The mixture obtained under a) was added to a solution of
500 mg (0.71 mmol) of 5-(2'-cyanovinyl)mesoporphyrin dimeth-
ylester nickel complex obtained in Step C of example 1 in 20
ml chloroform. The reaction was followed with TLC using a

10   mixture of 1% methanol in dichloromethane as the eluens. Af-
ter 16 hours of stirring at room temperature an aqueous solu-
tion of sodium acetate was added until the pH was 7 and the
reaction mixture was stirred until hydrolysis was complete.
Then the chloroform was removed by distillation under reduced

15   pressure, the solid mixture obtained in this way was filtered
off and dissolved in dichloromethane. With silicagel flash
chromatography a complex mixture of formylated products (350
mg, 0.48 mmol, 67 %) was obtained using 2% methanol in di-
chloromethane as the eluens. When using 5% methanol in di-

20   chloromethane a green fraction was collected which contained
45 mg of an impure mixture containing quino-annulated porphy-
rins as recognized from the v-shaped spot on the TLC obtained
with 80% THF and 20% diethylether (v/v). This latter fraction
was purified over silicagel for a second time using a mixture

25   of 60% THF and 40% diethylether (v/v). This yielded 25 mg of
a mixture containing 8 and 9 in a 2/1 ratio.

30

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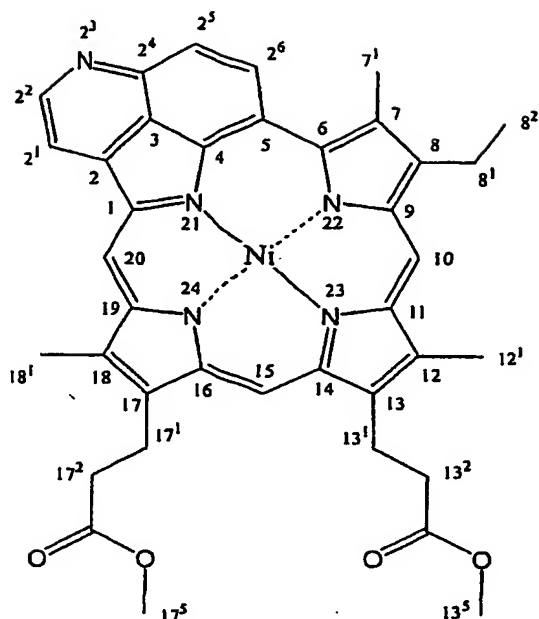
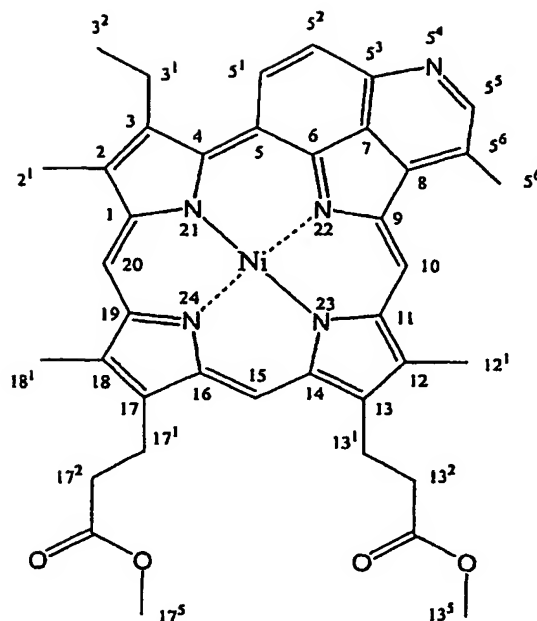(8)   (9)

Table 1

Overview of most of the compounds mentioned
in this application
(those of the schemes S1 – S4 are underlined)

1   mesoporphyrin dimethylester

2   mesoporphyrin dimethylester nickel(II)complex

3a   5-formylmesoporphyrin dimethylester nickel(II) com-
plex

3b   10-formylmesoporphyrin dimethylester nickel(II)
complex

3c   15-formylmesoporphyrin dimethylester nickel(II)
complex

3d   20-formylmesoporphyrin dimethylester nickel(II)
complex

4a   5-(2'-cyanovinyl)mesoporphyrin dimethylester
nickel(II) complex

4b   10-(2'-cyanovinyl)mesoporphyrin dimethylester
nickel(II) complex

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<u>4c</u>   15-(2'-cyanovinyl)mesoporphyrin dimethylester nickel(II) complex

<u>4d</u>   20-(2'-cyanovinyl)mesoporphyrin dimethylester nickel(II) complex

<u>5a</u>   quino[4,4a,5,6-efg]-annulated 7-demethyl-8-deethylmesoporphyrin dimethylester nickel(II) complex

<u>5b</u>   2'-methoxycarbonylquino[4,4a,5,6-jkl]-annulated 12-demethyl-13-de[2-(methoxycarbonyl)ethyl]mesoporphyrin dimethylester nickel(II) complex

<u>5c</u>   2'-methoxycarbonylquino[4,4a,5,6-qrs]-annulated 18-demethyl-17-de[2-(methoxycarbonyl)ethyl]mesoporphyrin dimethylester nickel(II)complex

<u>5d</u>   quino[4,4a,5,6-abt]-annulated 2-demethyl-3-deethylmesoporphyrin dimethylester nickel(II) complex

<u>6a</u>   quino[4,4a,5,6-efg]-annulated 7-demethyl-8-deethylmesoporphyrin dimethylester

<u>6b</u>   2'-methoxycarbonylquino[4,4a,5,6-jkl]-annulated 12-demethyl-13-de[2-(methoxycarbonyl)ethyl]mesoporphyrin dimethylester

<u>6c</u>   2'-methoxycarbonylquino[4,4a,5,6-qrs]-annulated 18-demethyl-17-de[2-(methoxycarbonyl)ethyl]mesoporphyrin dimethylester

<u>6d</u>   quino[4,4a,5,6-abt]-annulated 2-demethyl-3-deethylmesoporphyrin dimethylester.

<u>7a</u>   quino[4,4a,5,6-efg]-annulated 7-demethyl-8-deethylmesoporphyrin

7b   2'-methoxycarbonylquino[4,4a,5,6-jkl]-annulated 12-demethyl-13-de[2-(methoxycarbonyl)ethyl]mesoporphyrin

7c   2'-methoxycarbonylquino[4,4a,5,6-qrs]-annulated 18-demethyl-17-de[2-(methoxycarbonyl)ethyl]mesoporphyrin

7d   quino[4,4a,5,6-abt]-annulated 2-demethyl-3-deethylmesoporphyrin

<u>8</u>   quino[4,4a,5,6-bcd]-2-demethyl-3-deethyl-mesoporphyrin dimethylester nickel(II) complex

<u>9</u>   3'-methylquino[4,4a,5,6-efg]-7-demethyl-8-deethylmesoporphyrin dimethylester nickel(II) complex

<u>10</u>   5-(2',2'-dicyanovinyl)mesoporphyrin dimethylester nickel(II) complex

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    <u>11</u>   9'-aminocarbonylquino[4,4a,5,6-efg]-7-demethyl-8-deethylquinoporphyrin dimethylester nickel (II) complex

    <u>12</u>   9'-aminocarbonylquino[4,4a,5,6-efg]-7-demethyl-8-deethylquinoporphyrin dimethylester

    <u>13</u>   9'-aminocarbonylquino[4,4a,5,6-efg]-7-demethyl-8-deethylquinoporphyrin

    <u>14</u>   quino[4,4a,5,6-bcd]-2-demethyl-3-deethyl-mesoporphyrin dimethylester (derived from 8)

    <u>15</u>   quino[4,4a,5,6-bcd]-2-demethyl-3-deethyl-mesoporphyrin

    <u>16</u>   3'-methylquino[4,4a,5,6-efg]-7-demethyl-8-deethylmesoporphyrin dimethylester (derived from 9)

    <u>17</u>   3'-methylquino[4,4a,5,6-efg]-7-demethyl-8-deethylmesoporphyrin

    <u>18</u>   N-benzylquinolinium[4,4a,5,6-efg]-annulated mesoporphyrin dimethylester

    <u>19</u>   N-benzylquinolinium[4,4a,5,6-efg]-annulated mesoporphyrin

Table 2

Overview of mass spectrometry data (HR-FAB MS [M + H]) of some compounds from the examples

compound	Brutoformula	m/z (found)	m/z (calcd)
<u>2</u>	$^{12}C_{36}{}^{1}H_{41}{}^{14}N_4{}^{16}O_4{}^{58}Ni^+$	651.2469	651.2481
<u>3a-d</u>	$^{12}C_{37}{}^{1}H_{41}{}^{14}N_4{}^{16}O_5{}^{58}Ni^+$	679.2414	679.2430
<u>4a</u>	$^{12}C_{39}{}^{1}H_{42}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$	702.2593	702.2590
<u>4b</u> + <u>4d</u>	$^{12}C_{39}{}^{1}H_{42}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$	702.2585	702.2590
<u>4c</u>	$^{12}C_{39}{}^{1}H_{42}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$	702.2618	702.2590
<u>5a</u>	$^{12}C_{38}{}^{1}H_{36}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$	684.2108	684.2121
<u>5c</u>	$^{12}C_{38}{}^{1}H_{36}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$	684.2112	684.2121
<u>5d</u>	$^{12}C_{38}{}^{1}H_{36}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$	684.2149	684.2121
<u>6a</u>	$^{12}C_{36}{}^{1}H_{38}{}^{14}N_5{}^{16}O_4{}^+$	628.2921	628.2924
<u>6b</u>	$^{12}C_{36}{}^{1}H_{38}{}^{14}N_5{}^{16}O_4{}^+$	628.2912	628.2924
<u>6c</u>	$^{12}C_{36}{}^{1}H_{38}{}^{14}N_5{}^{16}O_4{}^+$	628.2933	628.2924
<u>6d</u>	$^{12}C_{36}{}^{1}H_{38}{}^{14}N_5{}^{16}O_4{}^+$	628.2869	628.2924
<u>8</u>	$^{12}C_{38}{}^{1}H_{36}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$	684.2134	684.2121
<u>9</u>	$^{12}C_{39}{}^{1}H_{38}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$	698.2283	698.2277
<u>10</u>	$^{12}C_{40}{}^{1}H_{41}{}^{14}N_6{}^{16}O_4{}^{58}Ni^+$	727.2548	727.2543

BIOLOGICAL EXPERIMENTS

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EXAMPLE 4

Figure 1 depicts the cell survival of Chinese hamster ovary
5   cells as measured with the standard MTT survival assay (Car-
michael, J., et al (1987), Cancer Research, 47, pp. 936 -
942). On the y-axis is plotted the percentage cell survival
as compared to a non-treated control, on the x-axis the con-
centration of the sensitizers 6a and 7a according to the in-
10 vention in micrograms per ml incubation medium. The cultured
cells were incubated with medium containing the sensitizer
for 4 hours. Thereafter the medium was replaced by phosphate
buffer and the cells were illuminated using broad band white
light (30 mW/cm^2) for 15 minutes. Subsequently the buffer was
15 replaced by fresh culture medium and the survival was as-
sessed 24 hours after the illumination.

EXAMPLE 5
To determine the acute toxicity, female CBA/CA mice were in-
20 jected intravenously over a period of 5 to 7 minutes with 10
mg/kg of bodyweight of a 1 mg/ml solution of 7a in polyethyl-
eneglycol 400: ethanol: water = 30 : 20 : 50 (by volume). All
mice survived the injection procedure and the period thereaf-
ter. There was no difference in behaviour or appearance of
25 the mice that received 7a (n = 3) or vehicle only (n = 3). In
addition, after pathological inspection, there was no differ-
ence in the macroscopic appearance of the major organs in
thorax and abdomen between the experimental and control mice.

30 EXAMPLE 6
Photodynamic therapy of mammals is impeded by the inadequate
properties of the porphyrins available in the art. Patients
treated must remain out of daylight for days after the treat-
ment because of the long half-life of porphyrins in the body.
35 Cases of severe damage to the blood vessels in which porphy-
rins were injected are known, and this is a serious drawback
to what could be a very important technique thanks to the lo-
calized treatment (by illuminating the sites to be treated).

25

Hairless mice were injected with compound 7a according to the
invention at 1 mg/ml. Clearance of this porphyrin derivative
was measured using the intrinsic fluorescence of the porphy-
rin. Using fiber optics (Y-shaped fiber), parts of the body

5   (thigh (th), to measure fluorescence in muscle, shoulder (sh)
to measure bone, ear to measure fluorescence in the skin and
liver and spleen (sp) to measure the fluorescence in these
organs) were illuminated with light (408 nm; through one
branch of the Y-fiber) to excite the Soret-band (note: with

10   photodynamic therapy absorption bands more to the red are ex-
cited), and fluorescence at 470 nm was measured (fluorescence
captured in the base of the Y-shaped fiber was guided to a
photo-detector via the second branch). Decay in the measured
fluorescence and the organ distribution strongly suggest that

15   7a is behaves as a compound that does not leave the blood
vessel.

Fig. 2 shows the fast clearance of the porphyrins according
to the invention (Y-axis: fluorescence in arbitrary units; X-
20   axis time in hours), promising to overcome the major disad-
vantage of Foscan® (mTHPC), which also has to be used at much
lower concentration to avoid acute toxicity. The urine of the
mice was coloured, also indicating rapid clearance from the
blood stream.

25